# 6 PRODUCT GEL FOR DETERMINING AMPLIFIED PRODUCT

## 6.1 TECHNICAL NOTES

6.1.1 The product gel is used to determine the success of the amplification process and for assessing the concentration of amplified DNA that should be loaded into the typing gel.

6.1.2 The 123 base pair ladder consists of 34 repeats of a 123 bp DNA fragment ranging in length from 123 to 4,182 bp.

6.1.3 Ethidium bromide (EtBr) is used to detect DNA by staining. It intercalates into the DNA molecule and fluoresces under UV light.

6.1.4 A UV transilluminator, at a wavelength of 302 nm, is used to visualize the fluorescent reaction between the EtBr and the DNA.

6.1.5 Primer-dimer bands and unincorporated primers may appear as broad bands in the lower molecular weight region of the gel.

## 6.2 EQUIPMENT

6.2.1 Pipette - 10 µL

6.2.2 Gel tank, cover and electrodes

6.2.3 Voltmeter

6.2.4 Power supply

6.2.5 Freezer, -20$^0$C

6.2.6 Microcentrifuge tube rack

## 6.3 MATERIALS

6.3.1 Agarose gel, (Refer to Appendix C)

6.3.2 Sterile ART tips - 10 µL

6.3.3 Microtiter plate

6.3.4 Gloves

6.4 REAGENTS

6.4.1 5X Loading buffer

6.4.2 123 bp Ladder

6.4.3 Ethidium bromide - 5 mg/mL (Optional - refer to Appendix C)

6.4.4 0.5X TBE buffer

6.5 PROCEDURE

6.5.1 Following the procedure outlined in Appendix C, prepare a product gel using 3.0% NuSieve agarose in 0.5X TBE buffer.

6.5.2 Load the product gel into the tank and add a sufficient volume of 0.5X TBE buffer to the tank to cover the product gel.

6.5.3 Add 2.5 µL of 5X loading buffer into a sufficient number of wells of the microtiter plate to correspond to the number of amplified samples that will be loaded into the product gel. Add 5 µL of the amplified sample to the appropriate well containing 5X loading buffer. Store the remainder of the sample at -20°C.

6.5.4 Load 1 µg of the 123 bp ladder plus 5X loading buffer into at least one well of the product gel (the 123 bp ladder and 5X loading buffer may be pre-mixed ahead of time). A total volume of 5 µL, which includes the 123 bp ladder and 5X loading buffer, should be loaded into the product gel. Actual volume of ladder must be determined based on concentration of stock 123 bp ladder.

6.5.5 Load the entire amount of sample from the microtiter plate into the designated well of the gel. Continue until all of the samples have been loaded.

6.5.6 Place the cover on the gel tank. Ensure that the gel tank is situated so that the red (positive) electrode is farthest from the loading wells.

6.5.7 Plug the red (positive) electrode into the positive plug of the power supply. Plug the black (negative) electrode into the negative plug of the power supply.

6.5.8 Turn on the power supply and set the voltage to the equivalent of 115 volts (using a voltmeter reading) for a minimum of 30 minutes, or until the loading buffer moves approximately one-fourth of the way across the gel.

**NOTE**: If ethidium bromide was not incorporated into the gel, the gel must be stained with ethidium bromide after electrophoresis. Soak the gel in 200 mL of 1X TBE and 40 µL EtBr for 20-30 minutes.

**WARNING:**

**ETHIDIUM BROMIDE IS MUTAGENIC. ALWAYS WEAR GLOVES WHEN HANDLING EtBr.**

6.5.9 When electrophoresis is complete, view the gel on a UV transilluminator or in a UV viewing cabinet. Following the steps outlined in Appendix D, Section 3.1 or 3.2, as appropriate, photograph the gel.

Option: The FMBIO II Fluorescent Image Analysis System may also be used to view and capture the product gel image. Refer to Appendix F, Fluorescent Detection of the Electrophoresis Gel-FMBIO II, for instructions.

6.5.9 Label the picture(s) with the FS Laboratory number and the examiner's initials and determine if the amplification of the sample(s) is complete by the visual appearance of bands in the gel. Under optimum conditions the amplified DNA for the PowerPlex® 16 BIO Systems should appear between the 123 bp and the 492 bp bands of the 123 bp ladder.

6.5.9.1 If a **WEAK** signal is observed (i.e., less than or equal to 0.05 ng/µL) using the AluQuant™ Human Quantitation System and **NO** amplified DNA is observed on the product gel, no further analysis will be conducted on this sample.

6.5.9.2 If a **STRONG** signal is observed using the AluQuant™ Human Quantitation System, but **NO** amplified DNA is observed on the product gel a dilution (i.e., 1:5) may be made of the original volume of the extracted DNA in order to reduce the level of any possible inhibitors. In addition, a higher concentration (i.e., 1.5 ng) of the isolated DNA may be used to enhance the possibility of obtaining amplified product from a highly degraded sample. Amplify these samples following the procedure outlined in Section 5, PCR Amplification, then repeat steps 6.5.1 through 6.5.9.

6.5.9.3 If **NO** signal is observed using the AluQuant™ Human Quantitation System and **NO** amplified DNA is observed on the product gel, no further analysis will be conducted on this sample.

6.5.10 If amplified product has been observed proceed to Section 7, Vertical Gel Electrophoresis.

♦END